PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/IB2005/002444	International filing date *(day/month/year)* 18.08.2005
Priority date *(day/month/year)* 18.08.2004	
International Patent Classification (IPC) or both national classification and IPC G01N21/64, G01N21/77	
Applicant UNIVERSITY OF BASEL	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2005/002444

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 12

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 12

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-11-13-18

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11,13-18
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-11,13-18
Industrial applicability (IA)	Yes:	Claims	1-11,13-18
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item IV

1.1 The application does not fulfil the requirements of the PCT with respect to unity of invention, i.e. two different subjects not linked by common inventive concept have been identified in the set of claims:

i) Subject 1 (claims 1 - 11, and 13 - 18) relates to an apparatus for detecting the fluorescence radiation emitted by target particles, excited by light emitted from a fibre tip, and collected by the same tip and to a corresponding method.

ii) Subject 2 (claim 12) also relates to an apparatus for detecting the fluorescence emitted by target particles and excited by light emitted from a plurality of parallel integrated optical waveguides and collected by them.

1.2 The common concept linking subject 1 with subject 2 is an apparatus for single molecule detection comprising
a light source disposed to generate excitation light on a first path, a dichroic mirror disposed in the first path of excitation light generated by the light source wherein the dichroic mirror is arranged to direct excitation light into a fibre aligner, an optical transducer coupled to the light source by the fibre aligner wherein the optical transducer comprises an optical waveguide made from a dielectric material having a first refractive index, a photon detector disposed to receive fluorescent back radiation, and a test solution having a second dielectric index lower than the first dielectric index wherein the test solution is disposed in a container and comprises one or more target molecules, wherein a tip of the optical waveguide is disposed in the test solution and excitation light is generated by the light source and transmitted by the optical waveguide so as to exit the tip, one or more target molecules are excited by the excitation light and wherein the optical waveguide is arranged to transmit fluorescent back radiation generated by the one or more target molecules along a second optical path to the photon detector detecting the transmitted fluorescent back radiation. The optical waveguide thereby provides a portion of both the first path of the excitation light and the second path of the transmitted fluorescent back radiation. The dichroic mirror is disposed also on the second path of transmitted fluorescent back radiation. A filter is disposed in the second path wherein the filter filters the

fluorescent back radiation. A lens is disposed on the second path that focuses the fluorescent back radiation for detection by the photon detector. A computer is connected to receive signals from the photodetector wherein the computer provides an interface for reading out of a measurement of detected photons of the fluorescent back radiation. The photon detector detects transmitted fluorescent back radiation from a single excited target molecule and the computer reads out measurement of detected photons generated by the single excited target molecule.

Such an apparatus is commonly known or rendered obvious by the prior art (see EP 0 383 912 wherein the digital multimeter is considered to comprise all essential features of a simple computer or can obviously be replaced by one, or the fluorometer described in the paper of Golden et al., Optical Engineering, vol. 31, no. 7, 1992, pages 1458 - 1462 which differs from the above apparatus by the mirror being a parabolic mirror with a pass hole; however, it is considered as a trivial and obvious measure to exchange this parabolic mirror by a dichroic mirror if desired and already indicated in the paper) such that the common concept linking subject 1 with subject 2 does not contribute to the prior art.

1.3 i) Subject 1 further relates to details of the fibre tip, to details of the optical coupling between target molecules and waveguide tip, and to a corresponding method.

ii) Subject 2 in addition relates to details of an integrated optical device including integrated parallel optical waveguides.

1.4 There thus does not exist a common concept linking subject 1 with subject 2 and contributing to the prior art which finds its expression also in the fact that there do not exist common or corresponding special technical features forming such a contribution. The requirements of the PCT for unity of invention thus are not fulfilled (Rule 13 PCT).

Re Item V

Reference is made to the following documents:

D1: GOLDEN J P ET AL: "FLUOROMETER AND TAPERED FIBER OPTIC PROBES FOR SENSING IN THE EVANESCENT WAVE" OPTICAL ENGINEERING, SOC. OF PHOTO-OPTICAL INSTRUMENTATION ENGINEERS. BELLINGHAM, US, vol. 31, no. 7, 1. July 1992, pages 1458-1462

D2: THOMPSON R B ET AL: "COMPONENT SELECTION FOR FIBER-OPTIC FLUOROMETRY" APPLIED SPECTROSCOPY, THE SOCIETY FOR APPLIED SPECTROSCOPY. BALTIMORE, US, vol. 44, no. 1. January 1990, pages 117-122

D3: EP-A-0 383 912 (TERUMO KABUSHIKI KAISHA) 29. August 1990

1.1 The application does not fulfil the requirements of the PCT because the subject-matter of claims 1 - 9 and 11, as far as they can be understood and construed, does not involve an inventive step in the sense of Article 33(3) PCT:

Document D1 (see figure 1 and page 1458, col. 2, last paragraph - page 1460, col. 1, paragraph 2) discloses an apparatus suitable for single analyte molecule detection comprising a light source (i.e. the Argon ion laser) disposed to generate excitation light on a first path, a mirror (the parabolic mirror) disposed in the first path of excitation light generated by the light source wherein the mirror is arranged to direct excitation light into a fibre aligner (see kinematic mount holding the fibre end), an optical transducer coupled to the light source by the fibre aligner wherein the optical transducer comprises an optical waveguide made from a dielectric material having a first refractive index (i.e. the fibre), a photon detector (i.e. the photodiode) disposed to receive fluorescent back radiation, and a test solution having a second dielectric index lower than the first dielectric index wherein the test solution is disposed in a container and comprises one or more target molecules wherein a tip of the optical waveguide is disposed in the test solution (see page 1460, col. 1, last paragraph - page 1460, col. 2, paragraph 1) and excitation light is generated by the light source and transmitted by the optical waveguide so as to exit the tip (see figure 1), one or more target molecules are excited by the excitation light and wherein the optical waveguide is arranged to transmit fluorescent back radiation generated by the one or more target molecules along a second optical path to the photon detector detecting the transmitted fluorescent back radiation. The optical waveguide thereby provides a portion of both the first path of the excitation light and the second path of the transmitted fluorescent back radiation. The mirror is disposed also on the second

path of transmitted fluorescent back radiation. A filter (see figure 1) is disposed in the second path wherein the filter filters the fluorescent back radiation. A lens is disposed on the second path that focuses the fluorescent back radiation for detection by the photon detector (see e.g. figure 1). A computer is connected to receive signals from the photodetector wherein the computer provides an interface for reading out of a measurement of detected photons of the fluorescent back radiation (see e.g. figure 1). The photon detector detects transmitted fluorescent back radiation from a single excited target molecule and the computer reads out measurement of detected photons generated by the single excited target molecule. The tip of the waveguide core has a fibre core configured to extend freely over a predetermined length (see figure 3). The photon detector detects transmitted fluorescent back radiation from a single excited target molecule. The transmitted fluorescent back radiation has a first wavelength and is generated primarily from excited target molecules located in a region of the test solution that is within one half of a first wavelength from the tip of the optical waveguide (see figure 3).

The apparatus of D1 differs from the apparatus of claims 1 - 9, and 11 in that the mirror is not a dichroic mirror but a parabolic mirror having a pass hole for the excitation light.

However, dichroic mirrors are well known alternatives to the parabolic mirror with pass hole in the field of fluorescence sensors with optical waveguide tips (see D1, page 1460, col. 1, paragraph 1, and D2, figures 1 and 2, and page 118, col. 1, paragraph 2 - page 119, col. 1, paragraph 1) such that the replacement of the parabolic mirror described in D1 by a dichroic mirror is considered as a trivial and obvious measure not involving an inventive step.

1.2 Using a simple cleaved fibre end instead of the single core extending from the fibre end is also well known in the art (see e.g. D3: figure 1, and page 18, paragraph 2 - page 20, paragraph 1) and despite the lower sensitivity is known to provide more robust sensor tips. It is therefore considered as obvious to replace the fibre sensor of D1 with a cleaved fibre end if a more robust tip end is desired. The subject-matter of claim 10 thus is considered to not involve an inventive step in the sense of Article 33(3) PCT.

2. The application does not fulfil the requirements of the PCT because the subject-matter of claims 13 -18 does not involve an inventive step in the sense of Article 33(2) PCT:

Document D1 (see the references cited above) describes a single analyte molecule detection method comprising the steps of:
- providing a an apparatus for single analyte molecule detection with a light source for generating excitation light, a mirror disposed on a first path of excitation light generated by the light source wherein the mirror directs excitation light into a fibre aligner, an optical transducer coupled to the light source by the fibre aligner wherein the optical transducer comprises an optical waveguide made from a dielectric material having a first dielectric index, and a photon detector disposed to receive fluorescent back radiation
- providing a test solution having a second dielectric index lower than the first dielectric index and comprising one or more target molecules wherein a tip of the optical waveguide is disposed in the test solution
- generating excitation light using the light source and transmitting the excitation light along a first path into the test solution using the optical waveguide
- exciting one or more target molecules in the test solution using the excitation light
- generating fluorescent back radiation using the one or more target molecules the transmitting the back radiation along a second path to the photon detector with the optical waveguide wherein the photon detector detects the back radiation
- filtering the transmitted back radiation using a filter disposed on the second path
- focusing the transmitted back radiation using a lens before the back radiation reaches the photon detector
- counting photons of the transmitted fluorescent back radiation detected by the photon detector
- sending signals to a computer that provides a read out interface that reads out and displays a measurement of detected and counted photons
- wherein the fluorescent back radiation transmitted by the optical waveguide has first wavelength and comprises substantially of fluorescent back radiation generated by one ore more target molecules located in a region of the test solution that is within one half of the first wavelength from the tip of the optical waveguide.

The method of D1 differs from the method of claims 13 - 18 in that the mirror is a parabolic mirror with pass hole and not a dichroic one. However, for the reasons already outlined above in detail with respect to claim 1, it is considered that the replacement of the parabolic mirror with a dichroic mirror is a trivial, obvious and straightforward measure for the skilled person which as such does not involve an inventive step.